Final Report for 2002 Federal-State Marketing Improvement Program

Project: Evaluation of Factors Effecting Survival During the Transportation of the Freshwater Prawn, *Macrobrachium rosenbergii*

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Executive Summary

The freshwater prawn is a commercially important culture species in the south central United States including Kentucky. Prawns are transported at high densities during two distinct phases of prawn production. To stock grow-out ponds juveniles are transported by truck in sealed containers. After harvest live adult prawns are transported by truck to urban Asian markets. Hauling stress has been implicated as a potential problem in both phases. Methodologies to reduce stress could contribute to increased industry viability.

The initial project evaluated the effect of water temperature, added substrate, salinity and stocking density on transport survival of market size prawn. Two temperatures (20 and 25C), with and without added substrate, and with and without added salt were evaluated in a 3X2X2 factorial. Each treatment combination was replicated in three 100 L insulated plastic containers, each containing 10 kg of adult market size prawn. The substrate consisted of 2.0 mm plastic mesh supported by a PVC frame. After 24 hours, water quality analysis was performed, all prawns were removed, determined to be alive or dead, weighed and counted. Regression analysis indicated no interaction between the main effects of substrate, salinity and water temperature on any measured variable. Substrate and salinity had no significant ($P<0.05$) impact on prawn survival. However, temperature and stocking density did significantly ($P<0.05$) affect survival. Prawn survival at 20 C averaged 96% compared to 24% at 25 C. Water temperature also significantly impacted total nitrite-nitrogen concentrations within transport containers, which averaged 0.58 mg/L at 20C and 0.93 at 25 C. Increased stocking density generally resulted in reduced survival ($P<0.05$); however, reduced water temperature allowed greater transport densities. These data indicate that while decreased water temperature positively impacts prawn survival and water quality in transport of freshwater prawns, added substrate and

increased salinity appears to provide no benefit. With the completion of this trial we successfully completed all the objectives as outlined in the grant proposal.

We also evaluated the efficacy of commercially available anesthetics for use with prawns and have identified some promising candidates for use under transport conditions. The use of anesthetics could possibly improve transport survival; however, to date anaesthetic agents have not been evaluated for use with prawns. In these studies we compared five anesthetics commonly used in finfish for efficacy on freshwater prawns. Anesthetics evaluated were tricaine (MS-222™), 2-phenoxyethanol, quinaldine, clove oil, and Aqui-S™. Each was evaluated at 100, 200, and 300 mg/L in three replicate 6 L glass containers containing five juvenile prawn. Relative sedation level was determined every three minutes for one hour; then recovery time was measured. Treatments were compared using non-parametric tests. Tricaine and 2-phenoxyethanol were determined to be ineffective on prawns at all rates tested. Clove oil induced anaesthesia faster and at lower concentrations than Aqui-S™ or quinaldine and Aqui-S™ induced anesthesia faster and at lower concentrations than quinaldine. At the highest treatment rate (300 mg/L) prawn suffered 60% mortality in the Aqui-S™ treatment, 20% mortality in the quinaldine treatment, and 0% mortality in the clove oil treatment. Additional research is needed to determine optimal time and dose relationships to minimize stress during harvest, handling, and transportation of the freshwater prawn.

Recently, a commercial scale verification trial was conducted; in which 1,000 lbs. of live prawn were purchased from local producers and transported to ethnic Asian markets in New York City for test marketing. The trial was a tremendous success with an estimated survival of >95% and wholesale buyers willingness to pay in excess of $7.00/lb live weight. Shipping at 16 C without added salt appeared to work the best; although all treatments appeared to be successful. Mortality of 10-15% from transport to final sale is considered acceptable in these markets. Major problems were holding conditions in the warehouse awaiting distribution to the retail markets. Holding problems were associated with lack of knowledge on holding facilities and personal experience with the species. In the future more familiarity with the species and their requirements will likely remedy this situation. Until better holding systems can be developed in the warehouse, time from arrival to the retail market to the consumer should be less than 24 hours. The estimated market demand in the greater New York area is conceivably 2000 lbs/week at a retail price of $11-13/lbs. It is possible that a higher price could be realized in the months of July and August when their exists a shortage of live marine shrimp in these markets.

Introduction

Prawn production in the United States has increased from less than 10 acres five years ago to more than 1,000 acres anticipated to be in production in 2002. According to National Marine Fisheries Market report the average price paid for 10-12 count shrimp ranged from $9.75-13.35 per pound for processed tails, with whole live animals bringing $12.00-20.00 per pound within the live and ethnic markets (based on preliminary data). However, addressing these markets for freshwater prawn has been constrained due to poor survival during transportation. Preliminary market research has identified an unfulfilled market potential in excess of 100,000 pounds per year for live freshwater prawns in Asian communities located in urban areas of the US and Canada. While the desire for the product remains strong, initial efforts to supply these markets have been hampered by poor prawn survival during transport and during wholesale and retail product distribution and display/holding. These problems are probably related to conditions during transport, which could be readily improved once proper environmental parameters for the species are identified.

No published studies have specifically addressed the effect of temperature or added substrate on the survival of market size prawn transported at higher densities (100g/L) which would be required to economically transport live prawn to distant markets (up to 24 hours). This study was designed to evaluate the effect of temperature and substrate on prawn survival and associated water quality variables within transport containers stocked at high densities.

Materials and Methods

Objectives 1-4 were completed using a similar research protocol. The experiments were designed as 2X2 factorials evaluating either temperature (20 and 25 C) and density; the presence of substrate and density, or salinity and density. The experimental system was designed to simulate transport conditions and consisted of insulated coolers each supplied with both a pure source of oxygen by an oxygen tank and atmospheric through a regenerative blower. Pure oxygen and air were supplied through separate 5.0 cm X 2.5 cm air stones in each container to maintain containers as close to oxygen saturation as possible. Each treatment was evaluated in three replicate 100 L insulated plastic containers per treatment (12 containers total) each containing either 5 or 10 kg of adult market size (average individual weight 47.5 ± 6.2 g) prawn. The substrate consisted of 2.0 mm plastic mesh supported by a PVC frame. After 24 hours, water quality analysis was performed, all prawns were removed, determined to be alive or dead, weighted and counted..

Data were analyzed by Two-way analysis of variance (ANOVA) using Statistix version 4.1 (Analytical Software, Tallahassee, Florida, USA) for survival and water quality variables. If ANOVA indicated significant treatment effects the Least Significant Difference test (LSD) was used to determine differences among means ($\underline{P} \leq 0.05$). All percentage and ratio data were transformed to arc sin values prior to analysis. Data are presented in the untransformed form to facilitate interpretation.

Results

Regression analysis indicated no interaction ($P>0.05$) between the presence of substrate, salinity or water temperature on any measured variable. Therefore, each variable was analyzed separately for their effect on water quality and prawn survival. Neither the presence of substrate or increased salinity had a significant impact ($P>0.05$) on any measured water quality parameter or prawn survival.

Water temperature had a highly significant ($P<0.01$) impact on prawn survival. Survival was significantly reduced ($P<0.01$) in the 25 C transport containers (24.3%) compared to those stocked at 20 C (96.48%). These differences are likely a result of the increased metabolic rate at the increased water temperature. There was no significant difference ($P>0.05$) in total ammonia-nitrogen, un-ionized ammonia-nitrogen, or pH between temperature treatments, which averaged 28.60 mg/L, 0.64 mg/L, and 7.62, overall. There was also no significant difference in measured dissolved oxygen concentrations due to the different transport temperatures; which averaged 3.73 mg/L for the 20 C containers and 2.39 mg/L for the 25 C containers. Nitrite-nitrogen was significantly higher ($P<0.05$) in 25 C containers (0.93 mg/L) compared to 20 C containers (0.63 mg/L); although, these values are not sufficient to cause stress in prawn.

Discussion

If efficient harvest, holding and transporting techniques can be developed, the potential exists for sales of large amounts of product in the live ethnic markets. However, wholesale buyers have experienced problems in transporting and holding live prawns including; poor survival in transport and in live holding facilities. Previous research reported acceptable transport densities for juvenile prawn are 10-25 g/L (Coyle et al. 2001). These densities are very low compared to reported densities for fin-fish 100-200 g/L and are not economically feasible for long distance transport (Jensen 1990). In this experiment, market size prawn were successfully (96% survival) held in transport containers for 24 hours by reducing the water temperature to 20 C. If these results are proven to be effective under field conditions this would allow higher transport densities which could potentially allow access into new markets for live product. This could have a positive impact on the economic viability of small farms in the region through increased export potential, production diversification, and an increased ability to supply consumers with new products of the highest possible quality. Field verification trials should be conducted under commercial conditions before recommendations are made to farmers.

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The following are projects that were conducted using FSMIP funds that were in addition to the original objectives described in the proposal:

Efficacy of Anaesthetics

Based on the results of the transport trials, and the territorial and cannibalistic nature of prawn, we decided to evaluate the efficacy of general anaesthetics on freshwater prawns. Anesthetics are used routinely when transporting fish to reduce stress and improve transport survival. The use of anesthetics could greatly improve transport survival in prawn; however, currently little is known about the use of anaesthetic agents with prawns.

We compared the efficiency of five anesthetics commonly used in finfish: Tricaine (MS-222™), 2-phenoxyethanol, quinaldine, clove oil, and Aqui-S™. Anesthetics were applied at 100, 200, and 300 mg/L in three replicate 6 L glass containers containing 5 juvenile prawn (0.7 g). Times to induction of, and recovery from, anesthesia were measured and compared between treatments. Tricaine and 2-phenoxyethanol were determined to be ineffective at all rates tested. Clove oil generally induced anaesthesia faster and at lower concentrations than Aqui-S™ or quinaldine. Aqui-S™ generally induced anesthesia faster and at lower concentrations than quinaldine. At the highest treatment rate (300 mg/L) prawn suffered 60% mortality in the Aqui-S™ treatment, 20% mortality in the quinaldine treatment, and 0% mortality in the clove oil treatment. Additional research to determine optimal time and dose relationships to minimize stress during transport should be conducted.

Commercial Verification Trial

Recently, a commercial scale verification trial was conducted; in which 1,000 lbs. of live prawn were purchased from local producers and transported to ethnic Asian markets in New York City for test marketing. The trial was a tremendous success with an estimated survival of >95% and wholesale buyers willingness to pay in excess of $7.00/lb live weight. Shipping at 16 C without added salt appeared to work the best; although all treatments appeared to be successful. Mortality of 10-15% from transport to final sale is considered acceptable in these markets. Major problems were holding conditions in the warehouse awaiting distribution to the retail markets. Holding problems were associated with lack of knowledge and experience with the species. In the future more familiarity with the species and their requirements will likely remedy this situation. Until better holding systems can be developed in the warehouse, time from arrival to the retail market to the consumer should be less than 24 hours (just in time delivery). The estimated market demand in the greater New York area is conceivably 2000 lbs/week at a retail price of $11-13/lbs. It is possible that a higher price could be realized in the months of July and August when there exists a shortage of live marine shrimp in these markets.

Potential Impact

If efficient harvest, holding and transporting techniques can be developed, the potential exists for sales of large amounts of product in the live ethnic markets. However, wholesale buyers have experienced problems in transporting and holding live prawns including; poor survival in transport and in live holding facilities. Previous research reported acceptable transport densities for juvenile prawn to be 10-25 g/L. These densities are very low compared to reported densities for fin-fish 100-200 g/L and are not economically feasible for long distance transport. In this experiment, market size prawn were successfully (96% survival) held in transport containers for 24 hours by reducing the water temperature to 20C. This could potentially allow access into new markets for live product.

Field verification trials have been conducted to evaluate these results under commercial conditions with positive results. This could have a positive impact on the economic viability of small farms in the region through increased export potential, production diversification, and an increased ability to supply consumers with new products of the highest possible quality. Due to the demand for live shrimp in ethnic Asian markets and their willingness to pay a premium ($12-14/kg) for live product, efforts to supply these markets are now being developed. Currently these markets are paying a 20-30% premium compared to traditional outlets for freshwater prawn, which could result in a significant increase in profit potential for prawn farmers in Kentucky and abroad.

Presentations

Coyle, S., J.H. Tidwell, D. Yasharian, and L.A. Bright. 2003. Comparative Efficiency of Anaesthetics for the Freshwater Prawn, Macrobrachium rosenbergii. Aquaculture America 2003, Book of Abstracts, February 18-21.

Danaher, J., T. Beavers, S. Coyle, L.A. Bright, D. Yasharian, and J. Tidwell 2003. Comparative Efficiency of Anaesthetics for the Freshwater Prawn, *Macrobrachium rosenbergii*. Thirteenth Biennial Research Symposium, Book of Abstracts, March 29-April 2.

Beavers, T., J. H. Tidwell, S. Coyle and D. Yasharian. 2003. Comparative Efficiency of Anaesthetics for the Freshwater Prawn, Macrobrachium rosenbergii. Kentucky Academy of Science Annual Meeting, Agricultural Sciences Section Abstracts, November 7-9. 1st Place Undergraduate Competition

Novello, N., S. Coyle, L.A. Bright, D. Yasharian and J. Tidwell. 2003. The effect of substrate and temperature on transport survival of the freshwater prawn, *Macrobrachium rosenbergii*. Thirteenth Biennial Research Symposium, Book of Abstracts, March 29-April 2.

Referred Journal Publications (Attached)

Coyle, S., J. Tidwell, T. Beavers, L.A. Bright, and D. Yasharian. (In press) Comparative efficiency of anaesthetics for the freshwater prawn, Macrobrachium rosenbergii. Journal of the World Aquaculture Society - Appendix 1

Coyle, S.D., J.H. Tidwell, D.K. Yasharian, and L.A. Bright. (In review) The effect of biomass density, temperature, and substrate on transport survival of market size freshwater prawn, Macrobrachium rosenbergii Journal of Applied Aquaculture – Appendix 2

Extension and Lay Publications

Yasharian, D., S. Coyle, J. Tidwell, and L.A. Bright. 2004. Evaluation of transport technologies for live hauling adult freshwater prawn, Macrobrachium rosenbergii. Kentucky Aquatic Farming Newsletter, 17(1):5-6.

Acknowledgments

We thank Wes Stilwell and Jason Danaher for assistance in data collection. This research was supported by USDA/FSMIP grant to the Kentucky Department of Agriculture for Kentucky State University under agreement number 12-25-G-0358.

2002 Prawn FSMIP - Expenditures

		Quantity	Price	Total
Objective 1	67.3 qt Insulated Containers	30	$179.00	$5,370.00
Objective 2	Titanium chiller 1/4 hp	3	$1,150.00	$3,450.00
Objective 3	Substrates	18	$160.00	$2,880.00
Objs 1,2,&3	1/4 hp circulation pump	10	$120.00	$1,200.00
Objs 1,2&3	Extra fine bubble diffusers	30	$52.00	$1,560.00
Objs 1,2&3	Flow regulators	30	$86.00	$2,580.00
Objs 1,2&3	Water quality supplies		$800.00	$800.00
Objs 1,2&3	Juvenile Freshwater Prawn for Transport Experiments	100,000	$0.03	$3,000
Objective 4	Live Market Size Freshwater Prawn for Marketing Research	2,000	$8.00	$16,000.00
Objective 4	Fiberglass Hauling Tanks	11	8106	$8106
Objective 4	Goose Neck Trailer	1	4625	$4625
Objective 4	Out of State Travel (New York)	1	1577.61	$1077.61
Objective 4	Regulators and Air Stones for Trailer	1	1910.10	$1910.10
	Total			$53,058.71

Appendix 1

The Effect of Biomass Density, Temperature and Substrate on Transport Survival of Market Size Freshwater Prawn, Macrobrachium rosenbergii

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ABSTRACT. After harvest, adult prawn are often transported for sales to live markets such as ethnic Asian outlets in major urban centers. Hauling stress and associated delayed mortality have hindered development and expansion of these markets. Methodologies to increase survival during transport could contribute to industry viability. Two independent trials were conducted. In the first trial, three biomass densities (25, 50 and 100 g/L) were evaluated in 100 L plastic containers with three replications per treatment. Water quality analysis were performed prior to stocking. After 24 hours water quality analysis were again conducted and all prawn were removed, determined to be alive or dead, weighed and counted. After 24 hours of simulated transport, there was no significant difference ($\underline{P} > 0.05$) between treatments for prawn survival; which averaged 98%, overall. Total ammonia-nitrogen and un-ionized ammonia-nitrogen concentrations increased ($\underline{P} < 0.05$) as biomass densities increased, though values remained within what are considered tolerable ranges. In the second trial, the effect of added substrate and temperature on transport survival was evaluated in prawn stocked at the high density (100 g/L). Two water temperatures (20 and 25C) with and without substrate were evaluated in a 2X2 factorial with three replicate 100 L insulated plastic containers per treatment combination (12 containers total). The substrate consisted of 2.0 mm plastic mesh supported by a PVC frame. Factorial analysis indicated no significant statistical interaction ($\underline{P} > 0.05$) between the presence of substrate and water temperature on any measured variable. The main effects of substrate and temperature were then analyzed separately. The presence or absence of substrate had no significant ($\underline{P} > 0.05$) impact on prawn survival. However, temperature did significantly ($\underline{P} < 0.01$) affect survival; prawn survival at 20C averaged 96% compared to 24% at 25C. These data indicate prawn can be successfully transported at 100 g/L for 24 hours at 20C. Adding substrate to the transport tank appears to provide no benefit.

KEYWORDS. Transport, Substrate, Temperature, Freshwater prawn, Macrobrachium

INTRODUCTION

Preliminary market research has identified a market potential in excess of 50,000 kg per year for live freshwater prawn in Asian communities located in urban areas of the United States and Canada (personal communication: Michael Lamb, Big Land Farm, Toronto, Canada). However, addressing these markets has been difficult due to poor survival during, and soon after, transport. It is thought that this is due to deteriorated water quality and predation in transport containers. If methodologies could be developed to increase transport, and post-transport, survival; this would allow producers in the region to address a relatively large market willing to pay in excess of $14/kg live weight.

Stocking density is one of the most important factors affecting survival and economics during transportation of freshwater prawn. Previous research reported acceptable transport densities for juvenile prawn are 10-25 g/L (Coyle et al. 2001). These densities are very low compared to reported densities for finfish 100-200 g/L (Jensen 1990), and are not economically feasible for long distance transport of market size prawn. However, relatively little research has focused on the survival of freshwater prawn during transport. Smith and Wannamaker (1983) successfully shipped 6 g juveniles in aerated plastic bags at 18 g/l for 24 hours and reported that neither increased salinity or increased substrate benefited survival. Harrison and Lutz (1980) reported survival of juvenile prawn increased when transported at reduced water temperature (20^{o}C). However, few if any studies have been conducted on the effects of different variables on the transport survival of market size prawn.

This study was designed to evaluate the effect of biomass density, water temperature and presence of substrate on survival of market size freshwater prawn subjected to simulated transport conditions and subsequent water quality variables for a 24 hour period.

MATERIALS AND METHODS

Water Quality

The water used in both experiments to fill transport containers was spring water which was heated to the appropriate treatment temperature. Water quality analysis was performed on each replicate container prior to conducting and immediately concluding each experiment. In both trials, water temperature and dissolved oxygen were measured using a YSI Model 58 oxygen meter (YSI Industries, Yellow Springs, Ohio, USA). Total ammonia-nitrogen and nitrite-nitrogen were measured using a DREL 2000 spectrophotometer (Hach Company, Loveland, Colorado, USA); pH was measured with a electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio, USA). Un-ionized ammonia was calculated as a percentage of total ammonia according to Boyd (1979).

Trial 1

This research was conducted at Kentucky State University's, Aquaculture Research Center, Frankfort, Kentucky. Ungraded, mixed sex, adult prawn (47.5 ± 3.2g) were obtained at harvest from research ponds. Approximately 50 kg were stocked into each of two 3,700 L fiberglass rectangular tanks. Prawn were fasted and held at 20°C with a flow rate of 60 L/min for one week prior to conducting experiments to ensure that all prawn were in a post-molt or "hardened" state. The first trial was primarily a range finding study to determine an appropriate biomass density to examine additional transport technologies. Three stocking densities (25, 50 and 100 g/L) were evaluated with three replications per treatment. Insulated 100L plastic coolers (HWL: 57 cm, 57 cm, and 144 cm, respectively) were used as "model" transport tanks. Each container was supplied with both pure oxygen from an oxygen tank and compressed air from a regenerative blower. These were delivered through separate 5.0 cm X 2.5 cm air stones in each container to maintain dissolved oxygen concentrations near saturation. After 24 hours, water quality analysis was performed, all prawn were removed, determined to be alive or dead, weighed and counted.

Trial 2

This study evaluated the effect of added substrate and temperature on post-harvest transport survival. The experiment was designed as a 2X2 factorial evaluating two temperatures (20 and 25C) and the presence of substrate (with and without). Each treatment combination was replicated three times (12 experimental units). The experimental system was the same as described for Trial 1. Each container was stocked with 10 kg of adult market size prawn (average individual weight 46.5 ± 6.2 g). The substrate consisted of 2.0 mm plastic mesh supported by a PVC frame with a 5 cm spacing between two layers, and was included at a rate sufficient to increase bottom surface area by 200% (1.6 m^2). Water quality analysis was performed prior to stocking and after 24 hours. After 24 hours, all prawn were removed, determined to be alive or dead, weighed and counted.

Statistical Analysis

In Trial 1, survival and water quality data were analyzed by analysis of variance (ANOVA) using Statistix version 4.1 (Analytical Software, Tallahassee, Florida, USA). If ANOVA indicated significant treatment effects, the Least Significant Difference test (LSD) was used to determine differences among means ($\underline{P} \leq 0.05$). For Trial 2, data were analyzed by the two-way analysis of variance to determine the effects of water temperature, presence or absence of substrate, and their interactions on water quality and prawn survival. Since factorial analysis indicated no significant statistical interaction ($\underline{P} > 0.05$) between the presence of substrate and water temperature on any measured variable. The main effects of substrate and temperature were then compared separately using Student's T-test. All percentage and ratio data were transformed to arc sin values prior to analysis (Zar 1984). Data are presented in the untransformed form to facilitate interpretation.

RESULTS AND DISCUSSION

For Trial 1, which evaluated prawn stocked at biomass densities of 25, 50 and 100 g/L, there was no significant difference ($\underline{P} > 0.05$) in prawn survival; which averaged 98% overall, after 24 hours. There was also no significant difference ($\underline{P} > 0.05$) in concentrations of dissolved oxygen, nitrite-nitrogen or pH between treatments which averaged 6.6 ± 0.63 mg/L, 0.3 ± 0.1 mg/L, and 7.7 ± 0.1, overall (Table 1). Total ammonia-nitrogen levels increased ($\underline{P} < 0.05$) as biomass densities increased in transport containers. Un-ionized ammonia nitrogen levels also increased ($\underline{P} < 0.05$) as biomass densities increased. However, ammonia concentrations did not reach lethal concentrations, primarily due to a relatively low pH and water temperature. Based on these data, it would appear that at higher pH, temperature and/or biomass densities (> 100 g/L) un-ionized ammonia levels could become limiting.

Trial 2 evaluated the effect of added substrate and temperature on transport survival. There was no significant interaction ($\underline{P} > 0.05$) between the presence of substrate and water temperature on any measured variable. This allows substrate and temperature to be analyzed separately for their effect on water quality and prawn survival. Substrate had no significant impact ($\underline{P} > 0.05$) on any measured water quality variable or prawn survival after 24 hours of simulated transport (Table 2). These data are in agreement with Smith and Wannamaker (1983), who reported that increased substrate did not benefit survival when they shipped 6 g juveniles in aerated plastic bags at 18 g/l for 24 hours.

In the temperature comparison, water temperatures in transport containers were maintained within 1C through the duration of the experiment. After 24 hrs of simulated transport, there was no significant difference ($\underline{P} > 0.05$) in total ammonia-nitrogen, un-ionized ammonia-nitrogen, or pH between temperature treatments, which averaged 28.6 mg/L, 0.6 mg/L, and 7.6 mg/L, overall (Table 3). There was a significant difference ($\underline{P} < 0.05$) in dissolved oxygen concentrations due to the different transport temperatures; which averaged 3.7 mg/L for the 20C containers and 2.4 mg/L for the 25C containers. Although some of this difference is directly related to the different solubilities of oxygen at the different water temperatures (Boyd 1979); the percent saturation of oxygen was also statistically different ($\underline{P} < 0.05$) for the two temperatures and averaged 43% for the 20C treatment and 30% for the 25C treatment (Table 3). Nitrite-nitrogen was significantly higher ($\underline{P} < 0.05$) in 25C containers (0.9 mg/L) compared to 20C containers (0.6 mg/L). Water temperature had a highly significant effect ($\underline{P} < 0.01$) on prawn survival; averaging 24% in the 25C transport containers compared to 97% in those maintained at 20C. This is in agreement with Harrison and Lutz (1980) who reported survival of juvenile prawn increased when transported at reduced water temperature (20^{o}C). These data indicate that water temperature appears to be the dominate factor affecting prawn survival during transport.

Within the optimal temperature range for most species, increases in transport temperatures significantly increases oxygen consumption and nitrogenous excretion (Chen and Kou 1996); therefore, reducing acceptable biomass densities or transport times. The optimum metabolic temperature range for M. rosenbergii is between 26 and 32^{o}C (Boyd and Zimmerman 2002). Rogers and Fast (1988) reported that M. rosenbergii are stressed at water temperatures below 22^{o}C. However, it appears that water temperatures of 20-21^{o}C may produce an anesthesia like state in prawn; which appears to be beneficial for transport and presumably for short term holding. The effect of water temperatures < 20-21^{o}C are not known, and should be evaluated to determine the effect on prawn survival and water quality parameters for short term holding and transport.

Strauss et al. (1991) determined that juvenile freshwater prawn could tolerate exposure to 2 mg/l un-ionized ammonia at a pH of 8.5 for up to 72 hours and that the toxicity of NH^3 decreases as M. rosenbergii juveniles increase in size and weight. Therefore, measured NH^3 concentrations were not considered lethal at the pH and temperature values measured in this study. New (1990) indicated that survival during the transport of M. rosenbergii is more closely related to decreased dissolved oxygen level than any other water quality variable. In this study, the dissolved oxygen concentrations in the 25C containers may represent stress conditions for freshwater prawn. M. rosenbergii are reportedly able to tolerate dissolved oxygen levels as low as 1 ppm for short time periods (Avault 1987), and become stressed at dissolved oxygen levels < 2 mg/L (Boyd and Zimmerman 2002). However, the cumulative effect of water quality degradation including marginally high ammonia and nitrite levels combined with low oxygen levels is not known. Several factors may contribute to increased oxygen demand for a limited period, such as high temperature, stress (such as crowded conditions in transport containers), high ammonia concentrations, or metabolism of feed (Neill and Bryan 1991). It is therefore likely that tolerance for low oxygen levels is reduced at relatively high concentrations of ammonia and nitrite such as was encountered in this study in the 25^{o}C treatment.

Previous research reported acceptable transport densities for juvenile prawn are 10-25 g/L (Coyle et al. 2001). These densities are very low compared to reported densities for finfish; 100-200 g/L (Jensen 1990), and would not be economically feasible for long distance transport. The results of this study indicate that market size prawn can be transported successfully for 24 hours at 100 g/L if water temperatures are reduced to 20C. If these results are proven to be effective under field conditions this would allow access into new markets for live product. Additional research should evaluate further reductions in transport temperatures (< 20^{o}C) and the incorporations of liquid oxygen and biofiltration as techniques to further increase acceptable biomass densities.

ACKNOWLEDGMENTS

Special thanks to Jason Danaher, Akua Larabi, Russell Neal and David Lowe for technical support throughout the study. This research was supported by a USDA/CREES grant to Kentucky State University under agreement KYX-80-91-04A and funding was provided by Kentucky's Regional University Trust Fund to the Aquaculture Program as KSU's Program of Distinction.

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Table 1. Prawn survival (%), dissolved oxygen (mg/L), total ammonia (mg/L), un-ionized ammonia (mg/L), nitrite-nitrogen (mg/L), and pH in transport containers stocked with market size freshwater prawn at 25, 50, and 100 g/L for twenty-four hours under simulated transport conditions. Values in the same row followed by different letters were significantly different ($P < 0.05$).

	Biomass Densities During Transport			
	Initial	25 g/L	50 g/L	100 g/L
Survival (%)	100	100 ± 0.0 a	97.5 ± 2.2 a	100 ± 0.0 a
Dissolved oxygen (mg/L)	8.3	6.7 ± 0.5 a	6.8 ± 0.9 a	6.1 ± 0.2 a
Total ammonia (mg/L)	0.32	2.1 ± 0.9 c	4.6 ± 1.6 b	15.7 ± 6.3 a
Un-ionized ammonia (mg/L)	0.00	0.0 ± 0.0 c	0.1 ± 0.0 b	0.3 ± 0.1 a
Nitrite-nitrogen (mg/L)	0.05	0.2 ± 0.0 a	0.3 ± 0.2 a	0.4 ± 0.1 a
pH	7.3	7.7 ± 0.1 a	7.8 ± 0.1 a	7.6 ± 0.1 a

Table 2. Main effect means* of prawn survival (%), dissolved oxygen (mg/L), dissolved oxygen (% of saturation), total ammonia (mg/L), un-ionized ammonia (mg/L), nitrite-nitrogen (mg/L) and pH in transport containers either with or without plastic mesh substrate after twenty-four hours stocked with market size prawn at 100 g/L under simulated transport conditions. *Means (±s.e.) of six replicate containers, means within a row followed by different letters were significantly different ($\underline{P} < 0.05$).

	Initial	With Substrate	Without Substrate
Survival (%)	100	58.8 ± 41.8 a	62.0 ± 41.5 a
Dissolved oxygen (mg/L)	7.1	3.2 ± 1.2 a	2.9 ± 1.2 a
Dissolved oxygen (% sat)	85.2	37.3 ± 13.8 a	36.8 ± 15.6 a
Total ammonia (mg/L)	0.2	27.1 ± 6.8 a	30.1 ± 6.2 a
Un-ionized ammonia (mg/L)	0.0	0.6 ± 0.3 a	0.7 ± 0.3 a
Nitrite-nitrogen (mg/L)	0.0	0.8 ± 0.2 a	0.8 ± 0.2 a
pH	7.3	7.6 ± 0.1 a	7.6 ± 0.1 a

Table 3. Main effect means* of prawn survival (%), dissolved oxygen (mg/L), dissolved oxygen (% of saturation), total ammonia (mg/L), un-ionized ammonia (mg/L), nitrite (mg/L) and pH in transport containers at two water temperatures stocked with market size prawn stocked at 100 g/L under simulated transport conditions for twenty-four hours. *Means (±s.e.) of six replicate containers, means within a row followed by different letters were significantly different ($\underline{P}$ < 0.05).

		Temperature	
		21 ^{o}C	26 ^{o}C
	Initial	Final	Final
Survival (%)	100	96.5 ± 2.1 a	24.3 ± 8.7 b
Dissolved oxygen (mg/L)	7.1	3.7 ± 1.3 a	2.4 ± 0.5 b
Dissolved oxygen (% sat)	85.2	42.8 ± 14.9 a	30.2 ± 6.4 b
Total ammonia (mg/L)	0.2	25.2 ± 1.6 a	32.0 ± 2.8 a
Un-ionized ammonia (mg/L)	0.0	0.4 ± 0.1 a	0.8 ± 0.2 a
Nitrite-nitrogen (mg/L)	0.0	0.6 ± 0.1 b	0.9 ± 0.2 a
pH	7.3	7.6 ± 0.1 a	7.6 ± 0.1 a

Appendix 2

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Comparative Efficacy of Anesthetics for the Freshwater Prawn, Macrobrachium rosenbergii

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Abstract

The freshwater prawn, Macrobrachium rosenbergii, is a commercially important culture species in the South Central United States. Two major constraints in the commercial culture of the freshwater prawn in the US are poor survival during live transportation of seed-stock to growout ponds, and live transportation of pond harvested prawn to distant live markets due to the territorial and cannibalistic nature of prawn. The use of anesthetics could possibly improve transport survival; however, to date anesthetic agents have not been evaluated for use with prawn. Two trials were conducted with juvenile freshwater prawn to compare the efficacy of anesthetics commonly used on fish. The first trial was designed to identify the most promising candidates. In Study 1, tricaine methanesulfonate (MS-222), 2-phenoxyethanol, quinaldine sulfate (quinaldine), clove oil, and Aqui-S™ were evaluated at 25 and 100 mg/L for one hour in three replicate 10 L glass containers, containing five juvenile prawn each. Relative sedation level was determined every three minutes for one hour; then recovery time and survival were measured. In Study 1, MS-222 and 2-phenoxyethanol were determined to be ineffective on prawn at all rates tested. Based on their performance in Study 1, quinaldine, clove oil, and Aqui-S™ were evaluated at 100, 200, and 300 mg/L in Study 2. Observations were determined as in Study 1. Clove oil and Aqui-S™ induced anesthesia faster and at lower concentrations than quinaldine. At the highest treatment rate (300 mg/L) prawn suffered 60% mortality in the Aqui-S™ treatment, 13% mortality in the quinaldine treatment, and 0% mortality in the clove oil treatment and control following a one hour exposure to these concentrations. Based on these data, Aqui-S™ and clove oil applied at 100 mg/L may be suitable anesthetic treatments for prawn. Additional research is needed to determine optimal time and dose relationships to minimize stress during holding, handling, and transportation of prawn.

The freshwater prawn, Macrobrachium rosenbergii, is a commercially important culture species in the South Central United States with an estimated 400 ha of pond production (Tidwell and D'Abramo 2003). Two major constraints in the commercial culture of the freshwater prawn in the United States are poor survival during live transportation of juvenile prawn from the nursery to grow-out ponds and post-harvest transportation of prawn to distant live markets (Coyle et al. 2001). Prawn are territorial and cannibalistic especially under crowded conditions; mortality during transport is thought to be largely due to negative interactions within prawn populations (Smith and Wannamaker 1983; Alias and Siraj 1988). This behavior limits carrying capacity under transport conditions to 10-20 g/L for prawn (Smith and Wannamaker 1983; Alias and Siraj 1988; and Coyle et al. 2001) compared to 40-600 g/L for various warm-water fish species (Jensen 1990).

Juvenile freshwater prawn (0.3-0.6g; D'Abramo et al. 1995) are transported from nursery facilities to production ponds for grow-out. Stress during this transport is thought to be the cause of undetected mortalities after prawn are stocked into ponds (Coyle et al. 2001). This can result in reduced pond stocking density, reduced pond production, poor feed conversion efficiency, and deteriorated water quality due to accumulation of uneaten feed. Survival in commercial ponds in temperate regions of the US is typically 50-60% in a 4-5 month growing season (Coyle and Tidwell 2003). However, survival in research ponds where prawn are nursed on-site and not transported to distant grow-out facilities is typically 85-90% (Tidwell et al. 1996; Tidwell et al. 1997; Tidwell et al. 1998; Tidwell et al. 1999; and Tidwell et al. 2000). Critical to the success of prawn farming in temperate climates is improved survival through pond grow-out. Reducing post-stress mortality following the transport of prawn juveniles from nursery facilities to grow-out ponds could greatly improve apparent grow-out survival.

The potential exists for sales of large volumes of live freshwater prawn in Asian communities located in urban areas of the US and Canada (Tidwell and D'Abramo 2000). In these regions, farm-gate prices for live freshwater prawn achieve US$ 13.2-22.0/kg (Tidwell and D'Abramo 2000); compared to the average price of approximately US $8.0/kg for imported processed shrimp (Harvey 2003). While the demand for live product remains strong, initial efforts to supply these markets have been hampered by poor prawn survival during transport, and during wholesale and retail product distribution and display. Poor survival has been attributed to deteriorated water quality and predation in transport and display containers (Coyle and Tidwell 2003). If methodologies can be developed to reduce stress during transport, a large market exists for live product in these urban areas.

The use of anesthetics has been reported to increase transport densities and duration in finfish (Summerfelt and Smith 1990). A number of anesthetics have been evaluated experimentally. Some of these anesthetics are illegal for use on food-fish in the United States; however, are used on non-food fish and in research; i.e.. quinaldine, 2-phenoxyethanol. Currently, the only anesthetics approved by the U.S. Food and Drug Administration (FDA) for use on aquaculture food products in the United States are tricane methanesulfonate (MS-222) and carbon dioxide (CO_2) (Schnick et al. 1986). Tricane has a 21-d withdrawl period before fish can be consumed and carbon dioxide may not produce sufficient anesthesia and has a relatively narrow margin for safety (Summerfelt and Smith 1990). Alternative anesthetics need to be developed for aquaculture that are cost effective, safe, efficacious and that could be registered by FDA for rested harvest and transport of live product for immediate sale as food.

Recently, much interest has been devoted to the study of clove oil as an anesthetic for fish. A solution of 10% clove oil mixed with 90% ethanol is reported to be an effective anesthetic at application rates between 25-150 mg/L for most fin-fish species (Soto and Burhanuddin 1995; Anderson et al. 1997; Taylor and Roberts 1999). Clove oil is 85-90% eugenol, with the remaining 5-15% made up of isoeugenol and methyleugenol. Eugenol is used as an anesthetic in human medicine and dentistry and is cleared for use as a topical anesthetic for use on humans by the FDA (Soto and Burhanuddin 1995; Nagababu and Lakshmaiah 1992). Although not approved as a new animal drug for general use as a fish anesthetic in the US, clove oil has been affirmed as Generally Recognized as Safe (GRAS) and can be added directly to human food (FDA 2002). Aqui-S™ is a relatively new product approved for use in aquatic species in New Zealand and Australia with 0-withdrawl time and is currently undergoing the New Animal Drug Act (NADA) approval process for use in the United States. The active ingredient in Aqui-S™ is iso-eugenol which is a component of clove oil. These anesthetics appear to have the greatest potential for approval for use with 0-withdrawal time and should be evaluated for their effectiveness for use as anesthetics in freshwater prawn.

To date, a limited amount of research has been conduced on the use of anesthetics in crustaceans. Brown et al. (1996) reported that MS-222 concentrations as high as 1000 mg/L had no effect on crayfish, Oronectes virilis. Ozeki (1975) found that crayfish, Procamburus clarki, exposed to eugenol, the active ingredient of clove oil, at doses of 200-1000 ppm were able to recover from the anesthetic effects with no mortality. Morgan et al. (2001) evaluated clove oil as an anesthetic for use on three Pacific coast crab species: dungeness crab, Cancer magister, hairy shore crab, Hemigrapsus oregonensis and northern kelp crabs, Pugettia producta, and indicated great differences in the application rates required to induce anesthesia for the different species. No references were found related to the use of anesthetics with the freshwater prawn, Macrobrachium rosenbergii.

The objectives of the present research were to evaluate the potential of five commonly used fish anesthetics for use with the freshwater prawn (Study 1), and then determine appropriate treatment concentrations for the most promising candidates (Study 2).

Materials and Methods

Study 1

Preliminary screening trials were conducted to identify candidate compounds and appropriate ranges of dose-concentrations for use with the freshwater prawn. Preliminary trials (Study 1) evaluated quinaldine sulfate (quinaldine) (Surelife Laroratories Corp., Seguin, TX, USA), clove oil (Sigma Chemical Co., St. Louis, MO, USA), Aqui-S^{TM} (AQUI-S New Zealand LTD, Lower Hutt, New Zealand), Tricaine methanesulfonate (MS-222) (Argent Chemical Laboratories, Inc., Redmond, WA, USA) and 2-phenoxyethanol (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA) at 25 and 100 mg/L.

Study 2

Based on their performance in the first trial, quinaldine, clove oil, and Aqui-S^{TM} were evaluated at 100, 200, and 300 mg/L in Study 2.

Experimental System

In both studies, there were 3 replicate aquaria per anesthetic treatment and 3 control aquaria (no anesthetic). In Study 1, the system consisted of thirty-three 10-L glass aquaria (including 3 control tanks) each filled with 6-L of municipal water that had been aerated for 24 hours in a 400-L plastic tank to remove chlorine. In Study 2, the system was the same except thirty aquaria were used including the 3 control tanks. Each aquarium contained an air stone supplied by a regenerative blower.

Application rates were based on the active ingredient for each compound. The compounds were applied to aerated containers 5 min. prior to stocking prawn to allow anesthetic agents to mix thoroughly. Five juvenile prawn (2.0g ± 0.1 for Study 1; 2.1g ± 0.1 for Study 2) were then randomly stocked into each aquarium.

The relative sedation of individual prawn were ranked based on observations made at 3-minute intervals. The rankings ranged from 0-2. Every 3 min all prawn in each aquarium were given tactile stimulation, by prodding with a plastic straw to stimulate a reaction, and observations were recorded for each prawn. A ranking of 0 was given to prawn that demonstrated no response to the anesthetic and a normal escape response to touch stimuli. A ranking of 1 was given to prawn that demonstrated a partial loss of equilibrium, but were still reactive to touch stimuli. A ranking of 2 was given to prawn demonstrating a complete loss of equilibrium and not reactive to stimuli.

The total duration of this study was 1 hour. If in any aquaria all the prawn were ranked at a sedation level of 2, they were moved into recovery tanks containing aerated freshwater for 1 hour. During recovery from anesthesia, the most apparent stage occurred when prawn first regained control of equilibrium and attained an upright position on the bottom of the tank. This was an unambiguous response and was adopted as the level of recovery for comparison among treatments.

Water quality analysis was performed using a YSI 85 oxygen meter (YSI, Yellow Springs, Ohio, USA) and Odyssey DR/2500 Spectrophotometer (HACH Company,Yellow Springs, CO, USA). Water quality conditions for Study 1 were: temperature, 24.0 ^{o}C; total dissolved oxygen, 8.3 mg/L; total ammonia-nitrogen, 0.34 mg/L; total nitrite-nitrogen, 0.002 mg/L; and pH, 7.3. Water quality conditions for Study 2 were: temperature, 23.7 ^{o}C; total dissolved oxygen, 8.5 mg/L; total ammonia-nitrogen, 0.29 mg/L; total nitrite-nitrogen, 0.002 mg/L; and pH, 7.5.

Statistical Analysis

For Study 1, experimental data consisted of 10 treatments (quinaldine, clove oil, Aqui-S^{TM}, MS-222 and 2-phenoxyethanol, each applied at 25 and 100 mg/L, respectively); (2) the blocking factor was time from 15 to 60 minutes, in 15 minute increments; and (3) each treatment pair (i.e., anesthetic and time) contained data from three replications, with each data point representing the observed level of anesthesia of juvenile freshwater prawn, based on the 0-2 index, averaged over five animals from the same aquarium undergoing an identical treatment, at all periods of time. Study 1 was performed as a preliminary range finding study to determine qualitative performance of the different chemical treatments in order to determine appropriate treatment concentrations and select the most appropriate chemicals for further evaluation. Statistical analysis was not performed on data for Study 1.

Experimental data from Study 2 were organized into the following dependent and independent variables: 1) proportion of prawn in Stages 0, 1,and 2 per aquarium (dependent), 2) dummy variables representing chemical treatments (quinaldine, Aqui-S^{TM}, and clove oil), 3) chemical concentration (100 mg/l, 200 mg/l, or 300 mg/l), and 4) time elapsed in minutes. If y_i represents the anesthesia state of a prawn (y_i = 0: no response, 1: partial loss of equilibrium, 2: complete loss of equilibrium), it can be interpreted as the observed effects of an underlying, continuous, latent variable (y^*_i), which symbolizes the unobserved level of anesthesia of prawn. If y^*_i were a linear function of the applied anesthetic, its concentration, and time, $y^*_i = \beta_0 * DVQ + \beta_1 * DVA + \beta_2 * DVC + \beta_3 * Conc + \beta_4 * Time + error = \boldsymbol{\beta}'\mathbf{X} + error$, where DVQ – DVC are dummy variables for quinaldine, Aqui-S^{TM}, and clove oil, respectively. Using the theory of ordinal discrete dependent variable regressions y is related to y* in the following manner: $y_i = 0$ if $y^*_i \leq 0$, $y_i = 1$ if $0 < y^*_i \leq \mu$, and $y_i = 2$ if $y^*_i > \mu$, where μ is a threshold parameter (Greene 1990). Although individual data for the dependent variable was unavailable, proportional data (as described above) were sufficient to develop an Ordered Probit regression model in LIMDEP 7 (Econometric Software Inc. 1998), that explained the variation in the proportion of prawn in different stages of anesthesia with respect to the independent variables described above.

Using the estimated β's from the above model, the probabilities of prawn being in the 3 stages of anesthesia were $P(y = 0) = 1 - \Phi(\beta'\mathbf{X})$, $P(y = 1) = \Phi(\mu - \beta'\mathbf{X})$, $P(y = 2) = 1 - \Phi(\mu - \beta'\mathbf{X})$, where Φ is the standard normal CDF. Using these probability formulae, one could derive the marginal effects of each regressor, i.e., $\partial P(y = i) / \partial x$, for I = 0, 1, and 2, which would clarify the effects of each independent variable on the likelihood of anesthesia of prawn.

Results and Discussion

Study 1

In Study 1, MS-222 and 2-phenoxyethanol were found to be ineffective for use as anesthetic agents with freshwater prawn at the treatment rates tested (Table 1). This is in agreement with Brown et al. (1996) who found that MS-222 concentrations as high as 1000 mg/L had no effect on crayfish (Oronectes virilis). Treatment rates of 25-100 mg/L with clove oil, Aqui-S™ and quinaldine proved to be only slightly effective at sedating prawn (Table 1); therefore, increased concentrations of these compounds (100, 200, and 300 mg/L) were evaluated in Study 2.

Study 2

In Study 2, quinaldine, clove oil, and Aqui-S^{TM} were all effective in immobilizing prawn. Induction times and levels of sedation were dose dependant and varied considerably between the three treatments (Table 2). The time required for induction of anesthesia was reduced as the concentration of anesthetics increased from 100 to 300 mg/L. Table 3. shows survival and recovery times for the different anesthetics and dose concentrations tested. Clove oil applied at 100, 200, and 300 mg/L required 18, 85, and 70 minutes, respectively, for full recovery, and resulted in 100% survival in all dose concentrations tested. Quinaldine applied at 100, 200, and 300 mg/L required; 12, 50, and 18 minutes for recovery and resulted in 100, 100, and 87% survival, respectively. Aqui-S^{TM} applied at 100, 200, and 300 mg/L required 28, 37, and 37 minutes for recovery and resulted in 93, 73, and 40% survival, respectively.

Results from the Ordered Probit regression show that all three anesthetics had significantly different effects on the anesthetic state of prawn. Table 4. shows that Aqui-S^{TM} and Clove oil had similar effects on the dependent variable, i.e., the marginal effects for both these chemicals significantly increased the likelihood of prawn reaching Stage 2 of anesthesia. However, in contrast, quinaldine had a substantially weaker impact on the anesthetic state of prawn. Marginal effects, computed at the mean values of other independent variables, show that quinaldine was only effective in causing prawn to reach Stage 1 of anesthesia. The marginal effects of other independent variables, such as chemical concentration and time, were predictable. The likelihood of prawn arriving at Stage 2 of anesthesia improved with higher chemical concentrations and additional time.

The above results are further illustrated in Figure 1. The three graphs in the figure show the progression of the latent variable y*, representing anesthesia of prawn, with respect to chemicals, and time. Here, Aqui-S™ and clove oil were kept at a low concentration (100 mg/l), and quinaldine was kept at a high concentration (300 mg/l), for purposes of comparison. Clearly, Aqui-S™ and clove oil were capable of making prawn reach Stage 2 within the first 30 minutes. The data also showed that, on average, quinaldine was not very effective, even at high concentrations, in causing prawn to reach Stage 2 of anesthesia.

Clove oil required less time to induction and required a longer recovery than Aqui-S™ and quinaldine, which could prove to be advantageous for transport. This is in agreement with Munday and Wilson (1997) who found recovery time after anesthesia with clove oil was two to three times longer in the coral reef fish, Pomacentrus amboinensis, than recovery from other chemicals. Additionally, prawn treated with clove oil resulted in 100% survival at the end of the recovery time for all treatment rates. Ozeki (1975) found that crayfish, Procamburus clarki, exposed to eugenol, the active ingredient of clove oil, at doses of 200-1000 mg/L were able to recover from the anesthetic effects with no mortality. Morgan et al. (2001) evaluated clove oil as an anesthetic for use on three Pacific coast crab species: dungeness crab, Cancer magister, hairy shore crab, Hemigrapsus oregonensis and northern kelp crab, Pugettia producta. Clove oil was the most effective in kelp crabs with induction times ranging from 54 to 2 min at concentrations of 15 to 250 mg/L. Dungeness crabs had induction times ranging from 68 to 16 min at 500 to 1500 mg/L, and shore crabs demonstrated the longest induction times (188 to 87 min) and required the highest concentrations (1000 to 3000 mg/L) to achieve immobilization. There appears to be significant variability within Crustacea on the induction times and concentrations required for anesthesia using clove oil. This may indicate that research should be conducted for each species before use. Although clove oil proved to be an effective anesthetic for use with prawn, it currently is not approved by the FDA for use on food fish or crustaceans in the United

States. In addition, the likelihood of clove oil being approved for use as an anesthetic is poor due to a lack of sponsorship, high composition variability between sources, and some constituents (isomers) have been shown to be carcinogenic (FDA 2002).

Aqui-S™ was found to be effective on freshwater prawn as a mild sedative at a rate of 100 mg/L. Although, when the prawn were treated with Aqui-S™ at 200-300 mg/L, significant mortality was observed during recovery. However, the application rates used in this study were very high compared to those recommended by the manufacturer for use on fish (10-25 mg/L). It appears that the safety margin is much greater for clove oil than for Aqui-S™ in prawn. However, Gardner (1997) recommended 125 mg/L clove oil or 500 mg/L Aqui-S™ as affective treatments for the humane killing of the Australian giant crab, Pseudocarcinus gigas, implying that greater concentrations of Aqui-S™ were required to kill P. gigas. Apparently, the efficiency of Aqui-S™ may be different for different species of crustaceans, as it is in different fishes. If a partial loss of equilibrium is found to be a suitable level of anesthesia, Aqui-S™ applied at 100 mg/L may prove to be effective for transport conditions. Clove oil and Aqui-S™ each have desirable traits for use in aquaculture: ease of use (relative to carbon dioxide), and calm induction to anesthesia. However, Aqui-S™ has the greatest potential for being approved for use in the US because it is currently undergoing the NADA process.

Based on the criteria of this experiment, quinaldine was not as effective as clove oil or Aqui-S™. In quinaldine treatments, total loss of equilibrium (rank 2) was not achieved in any of the levels tested (100-300 mg/L). Schoettger and Steucke (1972) reported that rainbow trout, Oncorhynchus mykiss, exposed to quinaldine, even at a state of total loss of equilibrium (stage 2 in this study), retained a strong reflex response to being touched. Summerfelt and Smith (1990) listed a poor suppression of reflex actions by anesthetized animals and irritability to mucous membranes as some of the major drawbacks of quinaldine. In the present study, prawn exposed to quinaldine exhibited muscle spasms and attempted to escape the tank. In contrast, prawn exposed to clove oil and Aqui-S exhibited a much calmer response to anesthesia. Munday and Wilson (1997) reported that a coral reef fish, Pomacentrus amboinensis, exposed to quinaldine swam rapidly and attempted to jump out of the tank when exposed to the bath treatment. If total loss of equilibrium is not necessary, 100-200 mg/L of quinaldine may be an effective anesthetic for prawn. Quinaldine is not approved by the FDA for use on food fish in the US and is reported to be carcinogenic (Summerfelt and Smith 1990). However, quinaldine is legal for use in some countries, and may be useful for sedating brood prawn in hatchery operations.

The properties required of an anesthetic vary with the procedure or objective. In this study the desired effect was total sedation, primarily to compare the relative effectiveness and margins of safety for the different anesthetics. A quick induction of anesthesia is desirable in most cases. In practice, a total loss of equilibrium may not be desirable in transport because overcrowding of sedated prawn at the bottom of the transport tank could result in asphyxiation. Under transport conditions, the sedation level 1 used in this study would likely be more appropriate. Therefore, clove oil or Aqui-S™ applied at 100 mg/L may be the best options when transporting prawn. The results of this experiment will be used to determine effectiveness of different concentrations of these anesthetics in improving prawn survival under actual transport trials. However, at this time there is no chemical anesthetic that is approved by the FDA for use with 0-withdrawal in the US. For producers to use anesthetics for transporting live prawn to market a 0-withdrawal anesthetic approved by FDA under NADA will be necessary.

Acknowledgments

We thank Wes Stilwell and Jason Danaher for assistance in data collection. This research was supported by USDA/CREES grant to Kentucky State University under agreement KYX-80-91-04A. Funding was also provided by Kentucky's Regional University Trust Fund to the Aquaculture Program as KSU's Program of Distinction.

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Table 1. The percent relative rank of freshwater prawn, Macrobrachium rosenbergii, exposed to different anesthetics applied at either 25 or 100 mg/L where: P0 = prawn which demonstrated no response to the anesthetic and a normal escape response to touch stimuli, P1 = prawn which demonstrated a partial loss of equilibrium, but were still reactive to touch stimuli, and P2 = prawn demonstrating a complete loss of equilibrium and not reactive to stimuli. These are actual observations made at 15 minute intervals.

Anesthetic	mg/L	15 min P0/P1/P2	30 min P0/P1/P2	45 min P0/P1/P2	60 min P0/P1/P2
Control	0	100/0/0	100/0/0	100/0/0	100/0/0
Clove Oil	25	100/0/0	100/0/0	100/0/0	93.3/6.7/0
Clove Oil	100	100/0/0	100/0/0	100/0/0	86.7/13.3/0
Aqui-S™	25	100/0/0	100/0/0	100/0/0	100/0/0
Aqui-S™	100	66.6/33.3/0	0/86.7/13.3	0/86.7/13.3	0/86.7/13.3
Quinaldine	25	100/0/0	60/13.3/26.7	26.7/13.3/60	0/20/80
Quinaldine	100	100/0/0	0/100/0	0/100/0	0/100/0
MS-222	25	100/0/0	100/0/0	100/0/0	100/0/0
MS-222	100	100/0/0	100/0/0	100/0/0	100/0/0
2-Phenoxyethanol	25	100/0/0	100/0/0	100/0/0	100/0/0
2-Phenoxyethanol	100	100/0/0	100/0/0	100/0/0	100/0/0

Table 2. The percent relative rank of freshwater prawn, Macrobrachium rosenbergii, exposed to different anesthetics applied at either 100, 200, or 300 mg/L where: P0 = prawn which demonstrated no response to the anesthetic and a normal escape response to touch stimuli, P1 = prawn which demonstrated a partial loss of equilibrium, but were still reactive to touch stimuli, and P2 = prawn demonstrating a complete loss of equilibrium and not reactive to stimuli. These are actual observations made at 15 minute intervals.

Anesthetic	mg/L	15 min P0/P1/P2	30 min P0/P1/P2	45 min P0/P1/P2	60 min P0/P1/P2
Control	0	100/0/0	100/0/0	100/0/0	100/0/0
Clove Oil	100	0/100/0	0/100/0	0/100/0	0/86.7/13.3
Clove Oil	200	0/100/0	0/46.7/53.3	0/13.3/86.7	0/0/100
Clove Oil	300	0/53.3/46.7	0/33.3/66.7	0/0/100	0/0/100
Aqui-S™	100	66.7/33.3/0	13.3/86.7/0	0/60/40	0/53.3/46.7
Aqui-S™	200	0/73.3/26.7	0/20/80	26.7/6.7/66.7	0/13.3/86.7
Aqui-S™	300	0/80/20	0/33.3/66.7	0/13.3/86.7	0/0/100
Quinaldine	100	100/0/0	60/33.3/6.7	40/53.3/6.7	26.7/60/13.3
Quinaldine	200	86.7/13.3/0	40/60/0	20/73.3/26.7	40/80/20
Quinaldine	300	33.3/66.7/0	0/100/0	6.7/73.3/26.7	40/80/20

Table 3. Time required to recover from anesthesia (Recovery Time) and survival of freshwater prawn, Macrobrachium rosenbergii, exposed to clove oil, Aqui-S™, and quinaldine when applied at 100, 200, and 300 mg/L. Values are means ± S.D. of three replicates. Means within a column followed by different letters are significantly different ($P<0.05$) by ANOVA.

Anesthetic	Rate (mg/L)	Recovery Time (min)	Survival (%)
Clove Oil	100	18.3 ± 2.9 de	100.0 ± 0.0 a
Clove Oil	200	85.0 ± 13.2 a	100.0 ± 0.0 a
Clove Oil	300	70.0 ± 25.0 ab	100.0 ± 0.0 a
Aqui-S™	100	28.3 ± 12.6 cde	93.3 ± 11.5 ab
Aqui-S™	200	36.7 ± 5.8 cd	73.3 ± 11.5 b
Aqui-S™	300	36.7 ± 20.2 cd	40.0 ± 32.7 c
Quinaldine	100	11.7 ± 7.6 e	100.0 ± 0.0 a
Quinaldine	200	50.0 ± 0.0 bc	100.0 ± 0.0 a
Quinaldine	300	18.3 ± 11.5 de	86.7 ± 11.5 ab

Table 4. Results of ordered probit regression of the proportion of prawn in the three stages of anesthesia. Data came from Study 2, with N = 141. Likelihood ratio index = 0.47.

Regressor	Coefficient estimate	Expected value / std. error	Marginal effects
DVA	-3.80	-1.78[a]	P(y=0): 0.64→0; P(y=1): 0.37→0.23; P(y=2): 0→0.77
DVC	-2.66	-1.02	P(y=0): 0.54→0; P(y=1): 0.46→0.01; P(y=2): 0→0.99
DVQ	-6.90	-2.71[b]	P(y=0): 0.34→0.21; P(y=1): 0.66→0.79; P(y=2): 0→0
DVA*Time	0.33	3.18[b]	_[c]
DVC*Time	0.30	2.73[b]	_[c]
DVQ*Time	0.34	3.03[b]	_[c]
Concentration	$0.51 * 10^{-2}$	2.01[b]	P(y=0): $-3.61 * 10^{-4}$; P(y=1): 1.59×10^{-4}; P(y=2): $2.01 * 10^{-4}$
$Time^2$	$-0.35 * 10^{-2}$	-2.57[b]	_[d]
μ	4.00	4.31[b]	

Table 4., continued

[a]P-value < 0.10.

[b]P-value < 0.05.

[c]Marginal effects for the “dummy variable*Time” regressors were incorporated into the marginal effects for the corresponding dummy variable regressors, evaluated at the mean time for the data sample.

[d]Marginal effects (ME) for time are dependent on the values of several regressors: $ME(P(y=0)) = -\phi(\beta'\mathbf{X})*[0.33*DVA+0.30*DVC+0.34*DVQ-0.70*10^{-2}*time]$; $ME(P(y=1)) = -[\phi(\mu - \beta'\mathbf{X}) - \phi(-\beta'\mathbf{X})]*[0.33*DVA+0.30*DVC+0.34*DVQ-0.70*10^{-2}*time]$; $ME(P(y=2)) = \phi(\mu - \beta'\mathbf{X})*[0.33*DVA+0.30*DVC+0.34*DVQ-0.70*10^{-2}*time]$, where ϕ is the standard normal PDF.

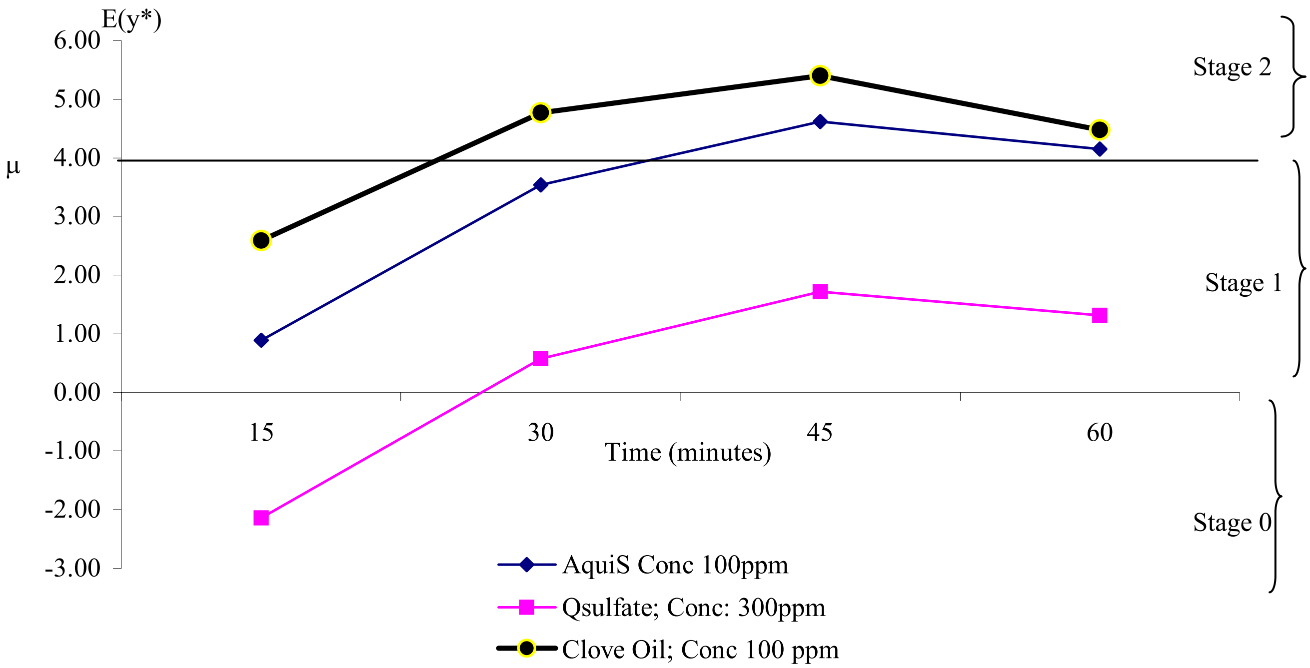
E(y*)
6.00
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
μ
15
30
45
60
Time (minutes)
Stage 2
Stage 1
Stage 0
AquiS Conc 100ppm
Qsulfate; Conc: 300ppm
Clove Oil; Conc 100 ppm

Figure Caption

Figure 1. Predicted stage of anesthesia of prawn, with respect to time, under three different anesthetics applied at different concentrations.